Biosci. Biotechnol. Biochem., 66 (7), 1552-1554, 2002

Note

α-Glucosidase Inhibitory Activity of a 70% Methanol Extract from Ezoishige (*Pelvetia babingtonii* de Toni) and Its Effect on the Elevation of Blood Glucose Level in Rats

Tomoki OHTA,[1,†] Shigefumi SASAKI,[1] Tadashi OOHORI,[1] Shuji YOSHIKAWA,[1] and Hideyuki KURIHARA[2]

[1]*Hokkaido Food Processing Research Center, Bunkyoudai-midorimachi, Ebetsu, Hokkaido 069-0836, Japan*
[2]*Graduate School of Fisheries Sciences, Hokkaido University, Minato, Hakodate, Hokkaido 041-8611, Japan*

Received September 20, 2001; Accepted February 19, 2002

The 70% methanol extract from ezoishige (*Pelvetia babingtonii* de Toni) inhibited the rat-intestinal α-glucosidase, sucrase and maltase activities, with IC_{50} values of 2.24 and 2.84 mg/ml. Sucrose was orally administered with or without the extract to rats at 1000 mg/kg. The postprandial elevation in the blood glucose level at 15 and 30 min after the administration of sucrose with the extract was significantly suppressed when compared with the control. These results suggest that the extract from ezoishige has potent α-glucosidase inhibitors and would be effective for suppressing postprandial hyperglycemia.

Key words: inhibitor; α-glucosidase; *Pelvetia babingtonii* de Toni

The frequency of diabetes, obesity, and hyperlipemia in the population worldwide is high and still increasing. In the course of these metabolic disorders, the concentrations of blood glucose, serum insulin, and lipoproteins each differ from the normal, especially after food intake. A reasonable way to control these carbohydrate-dependent diseases would be to limit intestinal carbohydrate digestion. Intestinal α-glucosidase (EC 3.2.1.20) plays an important role in carbohydrate digestion and absorption. Therefore, an inhibitor of intestinal α-glucosidase could be expected to retard carbohydrate digestion and absorption. Potent α-glucosidase inhibitors such as acarbose[1] and voglibose[2] have already been clinically used as medicines for diabetic and obese patients.

Great interest is currently being devoted to the physiological functions of food components relating to the prevention of diabetes and obesity. In previous *in vitro* studies, α-glucosidase inhibitors have been isolated from various food materials, *e.g.*, ougon,[3] hijiki,[4] tochu-cha,[5] welsh onion,[6] and clove.[7] However, the *in vitro* inhibitory activity is not always related to the *in vivo* activity for physiological action. To utilize food materials as physiological modulators, it is necessary to confirm the *in vivo* action of such materials following their oral administration.

In this present study, an extract of the brown alga, ezoishige (*Pelvetia babingtonii* de Toni), was examined for its *in vitro* inhibition of rat-intestinal α-glucosidase and its *in vivo* effect on the elevation of blood glucose in rats.

The *in vitro* α-glucosidase inhibition test was performed by using a crude α-glucosidase solution prepared from rat-intestinal powder. Rat-intestinal acetone powder was purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A). Sucrose and maltose were purchased from Kanto Kagaku Industry Co. (Tokyo, Japan), and ezoishige was collected from the coast of Mitsuishi, Hokkaido Prefecture in Japan. Ezoishige (100 g) was homogenized with 70% methanol (500 ml) by a Polytron device (Kinematica PT-6000, Littau, Switzerland) for 1 min at room temperature. The resulting homogenate was centrifuged at 10,000 × *g* for 10 min, and then the supernatant was passed through No. 5B filter paper. The filtrate was evaporated and dried under reduced pressure, before being dissolved in 70% methanol and used for the assay of α-glucosidase inhibitory activity. The inhibitory activity toward rat-intestinal α-glucosidase was measured by a slightly modified method of Asano *et al.*[8] One gram of rat-intestinal acetone powder was suspended in 10 ml of 0.9% saline, and the suspension sonicated (1 min × 3). After centrifugation (3,000 rpm × 30 min), the resulting supernatant was used for the assay. A crude α-glucosidase solution showed specific activities against maltase (1.94 units/mg of protein) and sucrase (0.42 units/mg of protein) which were measured by using sucrose and maltose as substrates. The assay mixture consisted of a 100 mM maleate buffer (pH 6.0, 0.7 ml), 500 mM sucrose or 500 mM maltose (0.1 ml), and the sample extract in 70% methanol (0–167.0 mg/ml, 0.1 ml). Methanol did not affect the enzyme activity under these conditions. The mixture

† To whom correspondence should be addressed. Fax: +81-11-387-4664; E-mail: tohta@foodhokkaido.gr.jp

was preincubated for 5 min at 37°C, and the reaction was initiated by adding a crude α-glucosidase solution (0.1 ml) to the reaction mixture. This mixture was incubated for 60 min at 37°C, the reaction being terminated by adding 1.0 ml of a 2.0 M maleate-Tris-NaOH buffer (pH 7.4). The glucose released in the reaction mixture was determined by a Glucose C-II Test Wako kit (Wako Pure Chemical Co., Tokyo, Japan) based on the mutarotase-glucose oxidase method. The reaction mixture (0.02 ml) and Glucose C-II Test Wako kit (3.0 ml) were mixed and incubated for 20 min at 37°C, before the absorbance of the mixture was measured at 505 nm.

Seven-week-old male Wistar strain rats were purchased from Nippon SLC Co. (Tokyo, Japan), rats weighing approximately 163–191 g being used. The animals were fed on standard feed (Labo MR stock, Nippon Nosan Kogyo Co., Tokyo, Japan) and tap water *ad libitum*. Each animal was housed in a cage under controlled temperature (22 ± 3°C) and humidity (50 ± 20%) with a cycle of 12 h for lights on and off. The animal experiments in this study were performed under the guidelines for animal experiments according to Notification No. 6 of the Japanese government.

The rats were used for the oral administration experiment after food deprivation for 12 h. The dried extract prepared as already described was dissolved in distilled water, and a sucrose solution (500 mg/kg) was orally administered to the rats with or without the extract (1,000 mg/kg) by using a zonde. Blood samples (0.5 ml) were collected before and 15, 30, 60 and 120 min after administration from the jugular vein of each rat under ether anesthesia. The plasma was separated from the collected blood, and the concentration of glucose was measured by the Glucose C-II Test Wako kit. The significance of differences in the glucose level against the control (sucrose alone) was analyzed by Student's *t*-test.

The results show that the 70% methanol extract from ezoishige had dose-dependent inhibitory activity against both sucrase and maltase with IC_{50} values of 2.24 and 2.84 mg/ml, respectively (Fig. 1). To evaluate the *in vivo* action, animal experiments were conducted with a single oral administration together with sucrose to the rats. Figure 2 shows the effect of the 70% methanol extract from ezoishige on the blood glucose level after orally administrating with sucrose to the rats. After the administration, the blood glucose level in the rats of the control group (sucrose alone) showed a maximum value after 15 min, before falling moderately after 30 min. The extract from ezoishige, however, significantly ($P<0.01$) depressed the postprandial elevation in blood glucose compared with the control group during the 15–30 min period after sucrose loading. The blood glucose level of the extract administered rats was identical to the level in control group during the

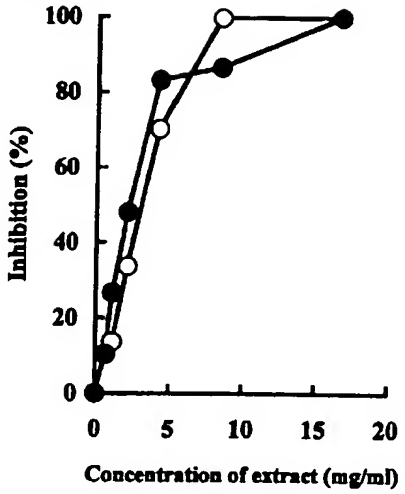

Fig. 1. Inhibitory Activity of the 70% Methanol Extract from Ezoishige against Sucrase and Maltase in Rat Intestine.
○ sucrase activity; ● maltase activity

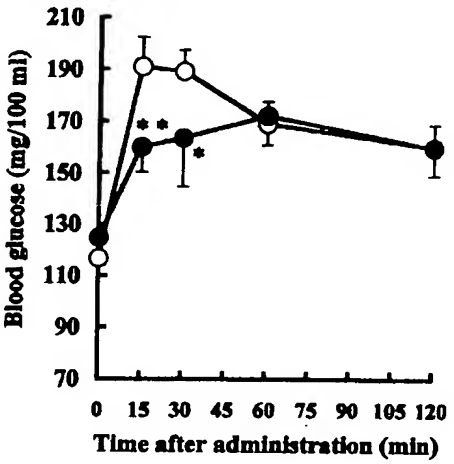

Fig. 2. Effect of the 70% Methanol Extract from Ezoishige on the Blood Glucose Level after an Oral Administration of Sucrose to Rats.

Rats were administered with sucrose (500 mg/kg) alone or with the extract (1,000 mg/kg). Each point represents the mean ± S.E. (n = 10). Significant difference in glucose level against that of the corresponding control: $^{*}P<0.01$, $^{**}P<0.001$.
○ sucrose; ● sucrose + extract

period from 60 to 120 min. These results show that the extract had a suppressive effect on the postprandial elevation in blood glucose after its oral administration to rats.

Although marine algae are increasingly being investigated for their novel and potentially bioactive components,[9] there have been no reports on the inhibitory activity of animal α-glucosidase and its *in vivo* action after an oral administration to rats. Most of the *in vitro* studies on food materials have used α-glucosidase from bakers yeast. Compared with the

previously reported inhibitory substances in foods[10] that have been evaluated *in vitro* by using yeast α-glucosidase, the extract obtained in this study showed the most potent inhibitory activity. Furthermore, the extract from ezoishige significantly suppressed the postprandial elevation in blood glucose after an oral sucrose loading in rats. The present results demonstrate that the extract from ezoishige contained potent α-glucosidase inhibitors and was effective for suppressing postprandial hyperglycemia. Ezoishige, which is an underutilized brown alga, is therefore considered to be a promising functional food material for controlling the blood glucose level to prevent and/or reduce the risk of diabetes and obesity. Purification and isolation of the α-glucosidase inhibitors in the extract from ezoishige are now in progress.

References

1) Schmidt, D. D., Frommer, W., Junge, B., Muller, L., Wingender, E., Trushelt, E., and Schafer, D., α-Glucosidase inhibitors. *Naturwissenschften*, **64**, 535–536 (1977).
2) Horii, S., Fukase, H., Matsuo, T., Kameda, Y., Asano, N., and Matsui, K., Synthesis and α-glucosidase inhibitory activity of *N*-substituted valiolamine derivatives as potential oral antidiabetic agents. *J. Med. Chem.*, **29**, 1038–1046 (1987).
3) Nishioka, T., Kawabata, J., and Aoyama, Y., Baicalein, an α-glucosidase inhibitor from *Scutellaria baicalensis*. *J. Nat. Prod.*, **61**, 1413–1415 (1998).
4) Kurihara, H., Ando, J., Hatano, M., and Kawabata, J., Sulfoquinovosyldiacylglycerol as an α-glucosidase inhibitor. *Bioorg. Med. Chem. Lett.*, **61**, 263–266 (1997).
5) Watanabe, J., Kawabata, J., and Niki, R., Isolation and identification of α-glucosidase inhibitors from tochu-cha (*Eucommia ulmoides*). *Biosci. Biotechnol. Biochem.*, **61**, 177–178 (1997).
6) Nishioka, T., Watanabe, J., Kawabata, J., and Niki, R., Isolation and activity of *N*-*p*-coumaroyltyramine, an α-glucosidase inhibitor in welsh onion (*Allium fistulosum*). *Biosci. Biotechnol. Biochem.*, **61**, 1138–1141 (1997).
7) Toda, M., Kawabata, J., and Kasai, T., α-Glucosidase inhibitors from Clove (*Syzgium aromaticum*). *Biosci. Biotechnol. Biochem.*, **64**, 294–298 (2000).
8) Asano, T., Yoshimura, Y., and Kunugida, K., Sucrase inhibitory activity of D-xylose and effect on the elevation of blood glucose in rats. *Nippon Eiyou Shokuryo Gakkaishi* (in Japanese), **49**, 157–162 (1996).
9) Fenical, W., New pharmaceuticals from marine organisms. *Trends Biotechnol.*, **15**, 339–341 (1997).
10) Matsui, T., Yoshimoto, C., Osajima, K., Oki, T., and Osajima, Y., *In vitro* survey of α-glucosidase inhibitory food components. *Biosci. Biotechnol. Biochem.*, **60**, 2019–2022 (1996).